

Instructions for Use

Periostin ELISA

Enzyme immunoassay for the quantitative determination of human periostin in serum/plasma, cell culture supernatants, nasal lavage fluid, vitreous fluid, tears and bronchoalveolar lavage fluid (BALF).

REF 30151132

 8x12

For illustrative purposes only.

To perform the assay the instructions for use provided with the kit have to be used.

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Periostin ELISA Kit (Human)

For research use only

Read this package insert before use.

1. INTRODUCTION

Periostin, also known as Osteoblast-specific factor 2 (OSF-2), is an extracellular matrix protein of approximately 90 kDa belonging to the fasciclin family¹. Periostin protein is composed of a cystein-rich region (EMI) and a four-coiled fasciclin-like repeats region (FAS1). Type I collagen and fibronectin have been found to bind to the EMI domain, Tenascin-C to the FAS1 domain². Periostin is upregulated by stimulation with Th2 cytokines (IL-4, IL-13)³. The periostin level correlates with both Th2-inflammation and fibrosis⁴. The concentration of periostin in biological samples is increased in many diseases including atopic dermatitis, asthma, scleroderma, cholangiocellular carcinoma and diabetic retinopathy⁵⁻⁹. It has also been suggested that periostin may serve as a companion diagnostic marker for asthma therapeutics¹⁰.

This ELISA kit specifically measures natural and recombinant human periostin. It does not react with either β ig-h3 or mouse periostin.

2. INTENDED USE

This kit is intended for quantitative determination of human periostin in serum/plasma, cell culture supernatants, nasal lavage fluid, vitreous fluid, tears and bronchoalveolar lavage fluid (BALF).

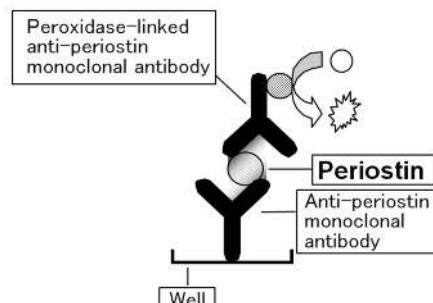
It is configured for research use only, and is not intended for either diagnostic or therapeutic use.

3. KIT COMPONENTS

Package	96 tests X 1	
Product Code	326070442	
①Antibody-coated Plate Anti-periostin monoclonal antibody	8 Well	X 12 Strips
②POD Conjugate (lyophilized) Peroxidase-linked anti-periostin monoclonal antibody	For 12 mL	X 1 Vial
③Standard (lyophilized) Recombinant human periostin	For 1.0 mL	X 1 Vial
④Positive Control (lyophilized) Human serum periostin	For 1.0 mL	X 1 Vial
⑤Sample Diluent	100 mL	X 1 Vial
⑥Conjugate Solvent	12 mL	X 1 Vial
⑦Color Reagent A 3, 3', 5, 5'-tetramethyl-benzidine	6 mL	X 1 Vial
⑧Color Reagent B hydrogen peroxide	6 mL	X 1 Vial
⑨Stop Solution	12 mL	X 1 Vial
⑩5X Wash Concentrate	100 mL	X 2 Vials
⑪Plate Seal	2 Sheets	

4. ASSAY PRINCIPLE

The assay employs a 2-step sandwich enzyme-linked immunosorbent assay (ELISA). The wells of the microtiter strips were pre-coated with a monoclonal antibody specific for periostin. Samples are added to the wells. Periostin in samples binds specifically to the immobilized antibody. Addition of POD-linked anti-periostin monoclonal antibody forms an antigen-antibody complex to which substrate solution is further added, into the wells, and color develops in proportion to the amount of periostin in the sample. The color development is stopped and the color intensity is then measured at 450 nm.



5. PROCEDURE

5-1. REQUIRED MATERIALS NOT PROVIDED IN THE KIT

- Micropipettes and tips (10,100,1000 μ L)
- Plastic tubes (for standards and samples preparation)
- 1 L measuring cylinder (for wash solution preparation)
- Vortex mixer
- Plate cover or plastic wrap
- Incubator at 25 °C
- Microplate washer
- 96-well microplate reader capable of measurement at 450 nm

5-2. REAGENT PREPARATION

Antibody-coated Plate Ready to use. Replace unused strips in the aluminum envelope with desiccant and store at 2-8 °C.

Sample Diluent Ready to use.

Standard Reconstitute Standard with 1.0 mL Sample Diluent. Mix gently and allow to stand for 10 minutes to ensure complete reconstitution. Prepare a standard dilution series using Sample Diluent and plastic tubes (see Table 1). Reconstituted and undiluted standard may be stored in plastic tubes in aliquots and will remain stable for 1 month below -30 °C. Avoid repeated freeze-thaw cycles. Make a standard dilution series for each assay.

Positive Control Reconstitute Positive Control with 1.0 mL Sample Diluent. Refer to the vial label for instructions. Mix gently and allow to stand for 10 minutes to ensure complete reconstitution. The concentration range of the positive control is indicated on the vial label. Reconstituted positive control may be stored in plastic tubes in aliquots and will remain stable for 1 month below -30 °C. Avoid repeated freeze-thaw cycles.

POD-conjugate solution Reconstitute POD-Conjugate with the entire volume of Conjugate Solvent. Mix gently and allow to stand for 10 minutes to ensure complete reconstitution. Reconstituted POD-conjugate solution may be stored in plastic tubes covered with aluminum foil as aliquots and will remain stable for 1 month below -30 °C. Avoid repeated freeze-thaw cycles.

Substrate solution Immediately before use, mix in a tube equal volumes of Color Reagents A and B, which have been brought to room temperature prior to use.

Stop Solution Ready to use.

Wash solution Dilute 5 x Wash Concentrate with four volumes of distilled water.

Table 1: Example of standard dilution method

Dilution method	Concentration (ng/mL)
S1: Dilute Standard with Sample Diluent to prepare 2 ng/mL diluted standard according to periostin concentration of vial label. (Total volume : 1 mL)	2.000
S2: Add 500 μ L of S1 to 500 μ L of Sample Diluent and mix.	1.000
S3: Add 500 μ L of S2 to 500 μ L of Sample Diluent and mix.	0.500
S4: Add 500 μ L of S3 to 500 μ L of Sample Diluent and mix.	0.250
S5: Add 500 μ L of S4 to 500 μ L of Sample Diluent and mix.	0.125
S6: Sample Diluent.	0

5-3. SAMPLE PREPARATION

Samples should be diluted with Sample Diluent using plastic tubes. For example, to prepare a 201-fold diluted serum sample, add 5.0 μ L of serum to 1.0 mL of Sample Diluent. Human periostin may be measured in serum, plasma, cell culture supernatants, nasal lavage fluid, vitreous fluid, tears and BALF. Aliquots of samples in plastic tubes can be stored at -80 °C. Avoid repeated freeze-thaw cycles.

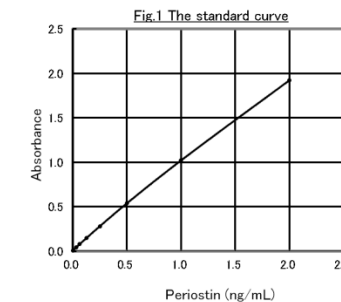
If samples contain periostin exceeding the highest standard, dilute with Sample Diluent in plastic tubes and remeasure.

5-4. PROCEDURAL NOTES

- Each reagent should be brought to room temperature at least 30 minutes before use.
- Though crystals may form in the microtiter wells, they do not affect assay performance. There is no need to wash the wells prior to use.
- A standard curve must be run with each assay.
- Use plastic tubes for diluting standards and samples.

5-5. ASSAY PROCEDURE

- Add 100 μ L diluted standards, positive control and diluted samples to wells.
- Cover all wells tightly with Plate Seal and incubate for 18 to 24 hours at 25 °C. Be careful not to spill liquids from the wells.
- Wash the wells 5 times with wash solution (>400 μ L/well) using an automated or manual microplate washer. After the final wash, turn over the plate and tap 4 or 5 times on a lint-free paper towel to remove any remaining wash buffer.
- Add 100 μ L of POD-conjugate solution to each well.
- Cover all wells tightly with Plate Seal and incubate for 90 minutes at 25 °C.
- Repeat the wash procedure as in step 3.
- Clean the backs of the wells. Be careful not to scratch the wells as this may interfere with measurements.
- Add 100 μ L of substrate solution to each well at a regular time interval. Incubate for 10 minutes at room temperature. During the reaction, cover the plate with a plate cover or plastic wrap to avoid contamination during the reaction.
- Add 100 μ L of Stop Solution to each well in the same sequence and time intervals.
- Read the absorbance of each well at 450 nm using a microplate reader within 60 minutes after addition of the Stop Solution. If wavelength correction is available, set between 550 and 700 nm.
- Read the periostin concentrations of unknown samples from the standard curve (see Fig.1) If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.



5-6. ASSAY SUMMARY

Prepare diluted standards, positive control and diluted samples.
 ↓
 Add 100 μ L samples to each well
 ↓ Incubate for 18-24 hours at 25 °C
 Wash wells 5 times.
 ↓
 Add 100 μ L POD-conjugate solution
 ↓ Incubate for 90 minutes at 25 °C
 Wash wells 5 times.
 ↓
 Add 100 μ L substrate solution
 ↓ Incubate for 10 minutes at room temperature
 Add 100 μ L Stop Solution
 ↓
 Read the absorbance at 450 nm (primary wavelength)
 550-700nm (secondary wavelength)

6. TEST PERFORMANCE

Dynamic Range	0.0 to 2.0 ng/mL
Detection Limit	20 pg/mL
Specificity	No cross-reaction with β ig-h3 No cross-reaction with mouse, bovine, canine periostin.
Reproducibility	Intra-assay coefficient of variation < 10% Inter-assay coefficient of variation < 10%
Recovery	80 to 120%

7. STORAGE

Expiration date is printed on the package. Store at 2-8 °C. Do not freeze.

8. NOTES

- This kit is for research use only. Do not use for either diagnostic or therapeutic purposes.
- Do not freeze the kit. Once it has been frozen, the kit must be discarded.
- Read the package insert and the latest SDS before use.
- Do not mix components from another lot. Do not use expired kits.
- Each reagent should be brought to room temperature at least 30 minutes before use.
- Strictly follow the methods for preparation of the reagent and the sample.
- As described in section 5-2. 'REAGENT PREPARATION', properly store aliquots of reconstituted standard solution, positive control and POD-conjugate solution. They will remain stable for 1 month, if stored below -30 °C. Avoid repeated freeze-thaw cycles. Replace unused strips in the aluminum envelope with desiccant. Store at 2-8 °C together with other reagents.
- A standard curve must be run with each assay.
- Handle samples with caution to avoid the possibility of infections.
- Sample Diluent contains a small amount of sodium azide. Sodium azide may produce an explosive azide metal, if contaminated with Pb or Cu. Wash with a copious volume of water to prevent azide accumulation upon disposal.
- Stop Solution is acidic (below pH 2). Therefore, care should be taken to avoid contact with skin and eyes.
- All reagents and samples should be regarded as potentially hazardous. Handle and dispose of samples with care. In case of contact with skin or mucosa, wash immediately with a copious volume of water. Consult a physician if any abnormalities develop.

9. REFERENCES

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


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	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lotto n.: / Lote N.º: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Da utilizzare entro:/ Usar até: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / Quantità dei tests: / N.º de Testes: / Αριθμός εξετάσεων:
	Concentrate / Konzentrat / Concentré / Concentrar / Concentrato / Concentrado / Συμπύκνωμα
	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizzato / Liofilizado / Λυοφιλοποιημένο
	In Vitro Diagnostic Medical Device / In-vitro-Diagnostikum / Appareil Médical pour Diagnostics In Vitro / Dispositivo Médico para Diagnóstico In Vitro / Dispositivo Medico Diagnostico In vitro / Equipamento Médico de Diagnóstico In Vitro / Ιατρική συσκευή για In-Vitro Διάγνωση
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	Store at: 2 - 8°C / Lagern bei: 2 - 8°C / Stocker à: 2 - 8°C / Almacene a: 2 - 8°C / Armazena a: 2 - 8°C / Conservare a: 2-8°C / Armazena em: 2-8°C / Αποθήκευση στους: 2-8°C
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	Distributor: / Distributor: / Distributeur: / Distributor: / Distributore: / Distribuidor: / Διανομέας:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Attenzione! / Cuidado! / Προσοχή!
	Symbols of the kit components see MATERIALS SUPPLIED. Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben. Voir MATERIEL FOURNI pour les symboles des composants du kit. Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS. Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT. Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS. Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.

Generic table, not all symbols are present in the product

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WARRANTY: The product is warranted to be free from material defects within the specific shelf life and to comply with product specifications delivered with the product. The product must be used according to the Intended use, all instructions given in the instructions for use and within the product specific shelf life. Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement.

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